ÄKTA*design*

Making your first run

Begin here with ÄKTApurifier 10/100

18-1120-02

Amersham Biosciences

Important user information

Meaning: Consult the instruction manual to avoid personal injury or damage to the product or other equipment.

WARNING!
The Warning sign is used to call attention to the necessity to follow an instruction in detail to avoid personal injury. Be sure not to proceed until the instructions are clearly understood and all stated conditions are met.

CAUTION!
The Caution sign is used to call attention to instructions or conditions that shall be followed to avoid damage to the product or other equipment. Be sure not to proceed until the instructions are clearly understood and all stated conditions are met.

Note
The Note sign is used to indicate information important for trouble-free or optimal use of the product.

Should you have any comments on this instruction, we will be pleased to receive them at:

Amersham Biosciences
SE–751 84 Uppsala
Sweden

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Adresses

Amersham Biosciences UK Limited
Amersham Place
Little Chalfont
Buckinghamshire
England HP7 9NA

Amersham Biosciences
SE-751 84 Uppsala
Sweden

Amersham Biosciences
800 Centennial Avenue
PO Box 1327
Piscataway
NJ 08855 USA

Contents

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1. About this guide

This guide is written for users who are not familiar with UNICORN™ software and ÄKTA™ purifier. Here you will learn the basics of UNICORN and how to operate ÄKTApurifier from UNICORN.

UNICORN is a software package for control and supervision of the ÄKTApurifier chromatography system. It runs on an IBM-compatible PC under Windows™ NT, and includes hardware for interfacing the controlling PC to the chromatography liquid handling parts of ÄKTApurifier

In this guide you will learn how to:

- create methods
- prepare the system for runs
- perform runs
- make simple evaluations
- make reports
- perform automatic method optimisation (Scouting)
- prepare automatically buffers of any pH (BufferPrep)

Follow the guide from page to page in front of the computer. The time will be well spent.

Note: To follow the instructions it is not necessary to read the comments (written with smaller font) containing additional information.

Pre-requisites

The system and the software must be installed and functioning and the monitor and pump calibrated as described in the separate Installation guide.

> **IMPORTANT!** Before using ÄKTApurifier, read all the safety information in Section 1.2 in ÄKTApurifier System Manual.

Typographical conventions

Menu commands and dialogue box prompts are identified in the text by **bold** text. A colon separates menu levels, thus **File:Open** refers to the **Open** command in the **File** menu.

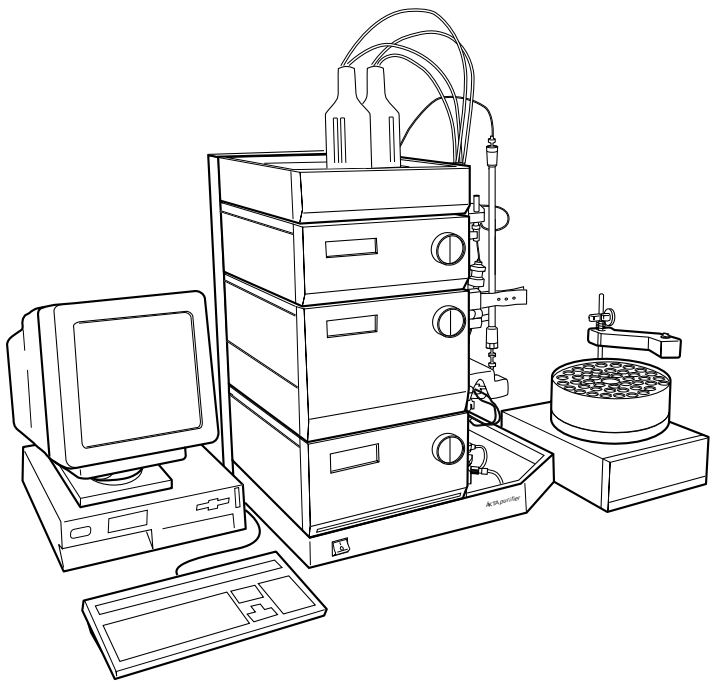
2. The system and the software

ÄKTApurifier

ÄKTApurifier is a fully automated liquid chromatography system designed for method development and research applications. The separation unit of the chromatography system has three main modules which are stacked on the left-hand side of a base platform. They are:

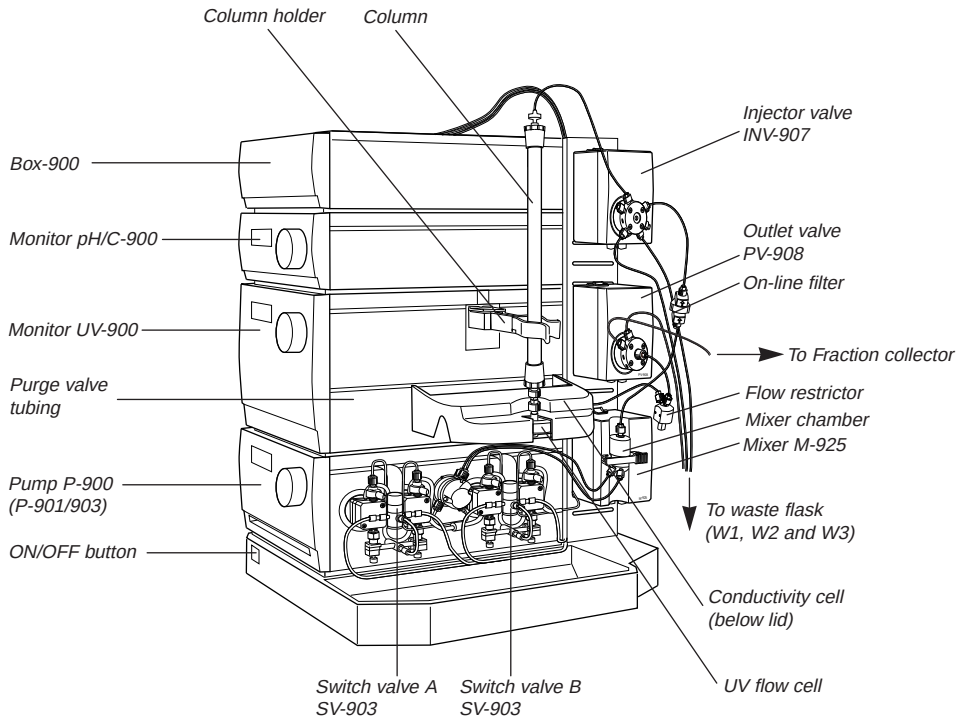
- Pump P-900, a family of binary high performance gradient pumps.
  - In ÄKTApurifier 100 the flow rate is up to 100 ml/min and the pressure up to 10 MPa (pump designation is P-901).
  - In ÄKTApurifier 10 the flow rate is up to 10 ml/min and pressure up to 25 MPa (pump designation is P-903).
- Monitor UV-900, a multi-wavelength UV-Vis monitor for simultaneous monitoring of up to 3 wavelengths in the range 190-700 nm.
- Monitor pH/C-900, a combined monitor for on-line conductivity and pH monitoring.

Fraction collector Frac-900 (optional) is placed to the right of the system. Up to 175 fractions can be collected.



Components, such as the mixer, column and different valves, are mounted in the section to the right.

The separation unit is controlled from UNICORN software.

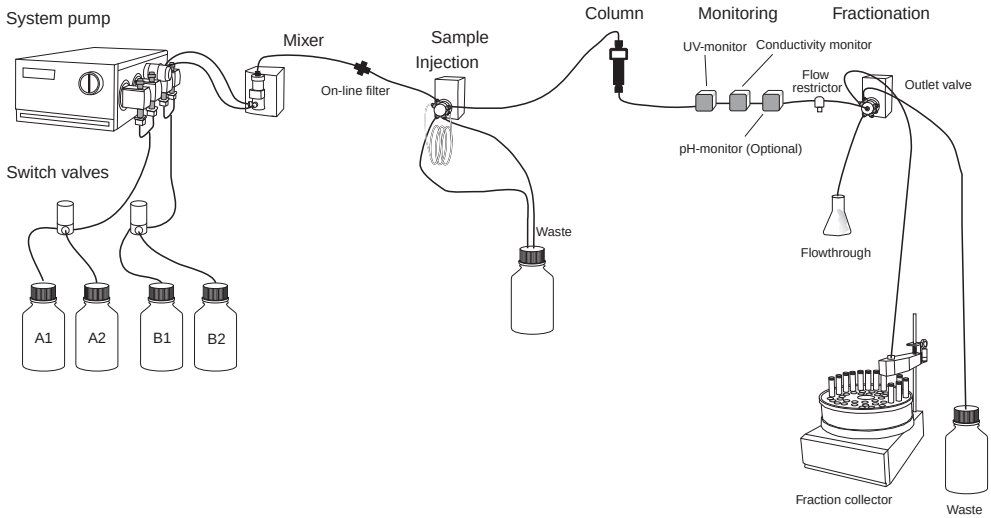


Pump P-900, Monitor UV-900 and Monitor pH/C-900 can also be controlled individually from the modules, without UNICORN software. In this guide, however, you will only learn how to operate the chromatography system from UNICORN.

Switch on the chromatography system with the ON/OFF button located on the front of the base platform to the bottom left.

Comment:

The flow path between the different components in the system is shown and described below. It is not necessary to go through this in detail to make your first runs. Look to the right-hand side of the system if you want to follow the description.



1. The pump has 4 pump heads, two for pump A and two for pump B. Pump A is the one closest to the front.
2. The buffer solutions are pumped through switch valves, and further to a mixer. Inlet A1 and B1 are placed in buffer A and B respectively. Inlet A2 and B2 are used when buffers are prepared automatically by BufferPrep.
3. The flow path continues from the pump to the mixer, and forward via an on-line filter to the injection valve.
4. A sample loop is connected between ports 2 and 6 on the injection valve. The sample loop is filled manually using a syringe; for this procedure, connect a fill port to port 3 on the injection valve.
5. After the injection valve, the flow is directed to the column, and then forward to the UV cell and the conductivity cell, which are located inside the cell holder on Monitor UV-900.
6. The flow path continues to the flow restrictor (and through the pH flow cell, if fitted (optional), and further to the outlet valve, which is used to switch the outlet flow between waste, fraction collection and flowthrough.
7. The flow path can continue to a fraction collector (optional), if desired.

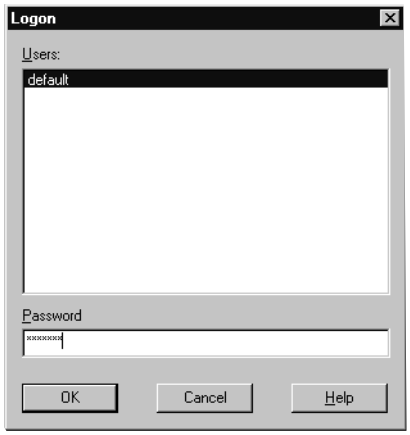
UNICORN overview

1 Switch on the computer. Log in to Windows NT 4 by first pressing **Ctrl-Alt-Del**, and then clicking on **OK**. After a while the Windows NT 4 desktop appears.

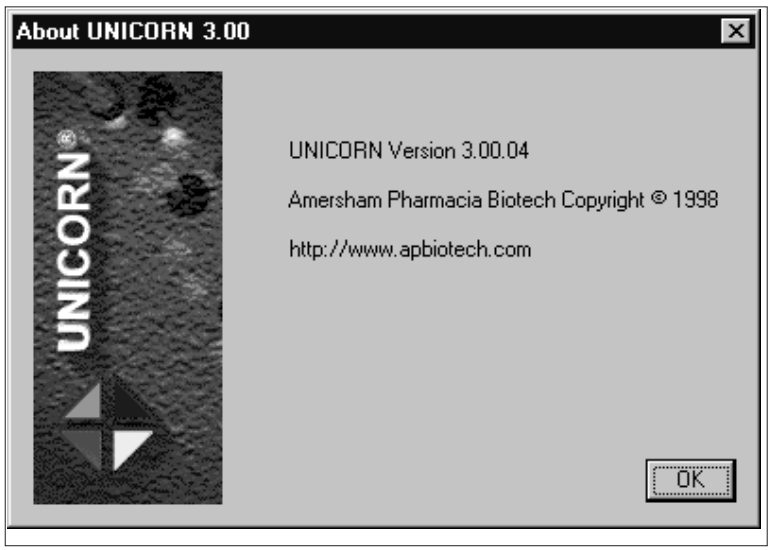
2 Start UNICORN by double-clicking on the UNICORN icon.

3 Select a user from the **Users:** list and enter the password. If you log in for the very first time, select user **default** and enter the password **default**. Click on **OK**.

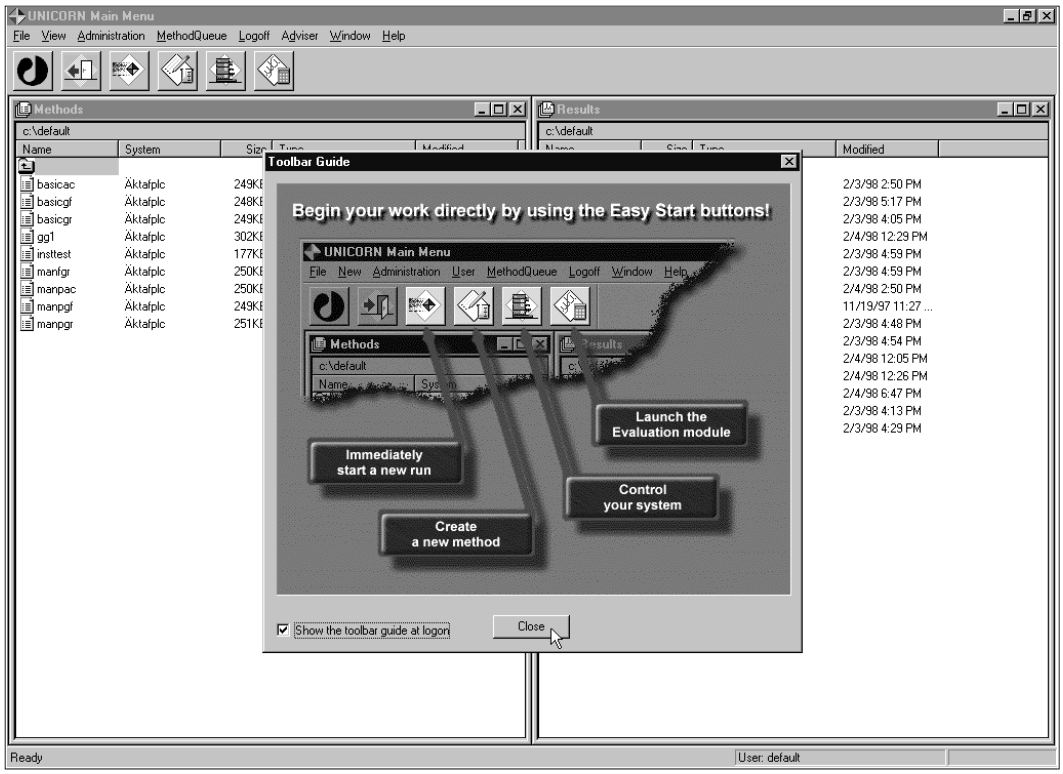
Note: *You should enter users and individual passwords before starting using ÄKTApurifier on a regular basis.*



4 An information window appears during start-up.



5 Eventually, the UNICORN Main menu window appears on the screen. At delivery, a Toolbar guide is displayed providing a quick guide on how to use the toolbar items. The Toolbar guide is inhibited by unchecking the square at the bottom left. Click on **Close**.



6 The Main menu window is the central part of UNICORN displays from which you navigate through the control system. It is mainly used for file handling. In the Methods window to the left in Main menu, all method files that you create are displayed. A method file contains a series of instructions for controlling a run. In the Results window to the right, all result files are displayed. A result file is the result from a run, including all documentation (e.g. the method used) and the generated chromatogram.

In general, UNICORN consists of 4 different modules of which the Main menu is one. The other modules are represented by icons in the Toolbar. These modules are:

- New method — opens a dialogue window for creating new methods.

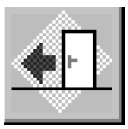
- System control — opens a dialogue window for controlling the system and running your methods.

- Evaluation — opens a dialogue window for evaluating your results.

A module is opened by clicking on its icon.

An additional three buttons are provided in the Toolbar. These are:

- Instant run — opens a dialogue window where you directly can choose a template method to run. This is handy for starting routine runs instantly.



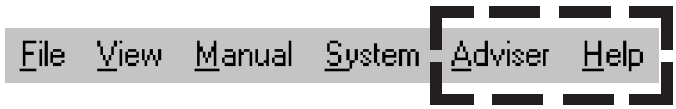
- Logon/Logoff — opens a dialogue window to control the logon/logoff process.

- about UNICORN — opens an information window about the UNICORN version and how to contact Amersham via the world wide web (internet).

Help and Adviser

Comprehensive on-line help is available. Click on the **Help** menu in the upper right corner of each module and select **Help for .....** to get general help about the current module and find new help topics, or **Help Index** for a specific topic. Double-click on green text to get more help. In any box, click on the **Help** menu to get help on how to use the current active box.

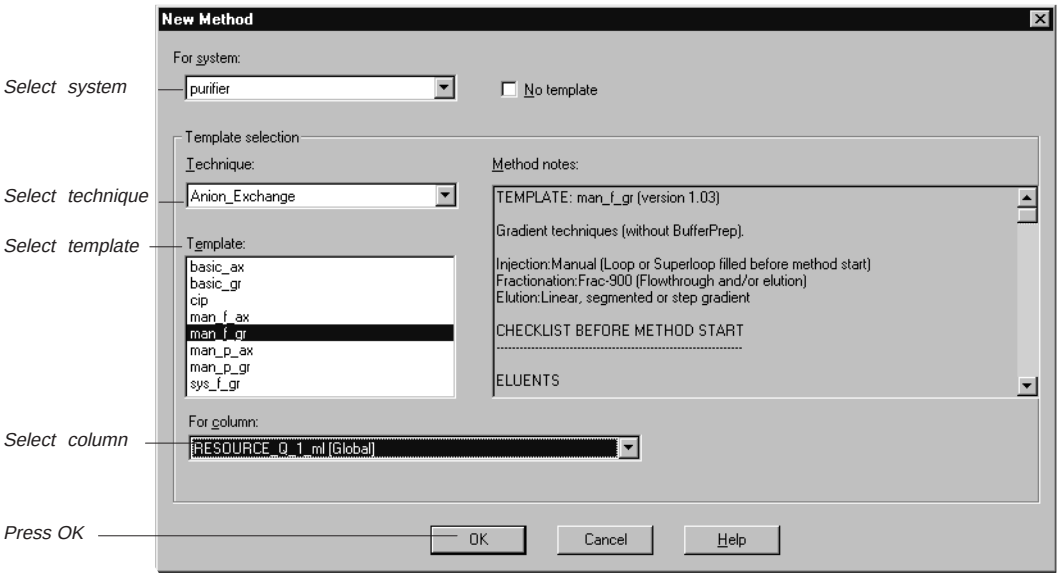
Click on **Adviser** in the menu bar, and choose the appropriate system to get help about separation optimisation protocols (Method Adviser), detailed information about chromatographic columns (Media Adviser), and information about ÄKTApurifier (System Adviser).



3. Creating a method

UNICORN is supplied with a number of almost ready-made methods called ***method templates***. Different method templates are available for different chromatographic techniques. The method templates can be run as they are or you can easily modify them to design your own method in a very short time. Let's start!

1 Click on  in the Main menu Toolbar. The New Method window will appear.



2 Select a system. Then select a chromatographic technique, for example **Anion_Exchange.**

3 A list of available templates will appear. By clicking on a template, an explanation for the template appears to the right in the "Method Notes" field. Select the template **Man_f_gr,** which is suitable for the first run.

Comment:
The other templates are briefly described at the end of this chapter.

4 Select the column you intend to use. The correct column volume, the recommended flow rate, and the correct pressure limit for that column will then be automatically implemented in the method.

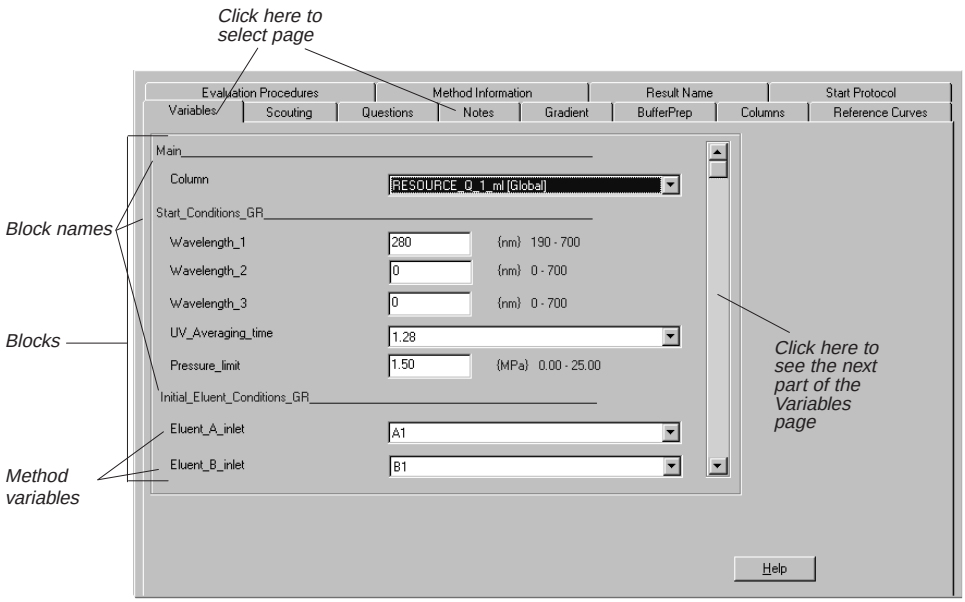
Comment:
If you manually alter the default values, and thereby exceed the recommended values for the selected column, you will get a warning when you save your method.

If you want to perform a test run without a column, you should still select a column (a small one is recommended) to get suitable default parameters in the method. Then in the method, use a peice of tubing to replace the column.

Comment:
If you do not find your column in the list, you can add one. See section 5.9 in UNICORN User Manual.

5 Click on **OK**. The method template will now be opened as an untitled method.

6 Select **View:Run setup** (may already be checked).



7 **Run setup** consists of a number of pages. You will only look at two of them now; **Variables** and **Notes**. You select a page by clicking on the respective tab at the top of the run setup screen.

8. On the **Variables** page, the method is presented by a number of **blocks** (name in blue). The **blocks** represent the typical steps in a chromatographic run:

   - Start conditions
   - Column equilibration
   - Flow through collection
   - Sample injection
   - Wash out unbound sample
   - Eluate fractionations

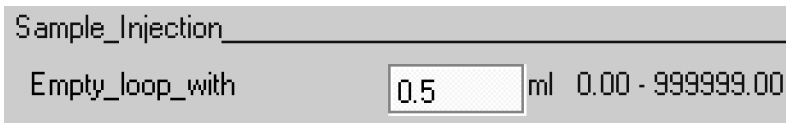
- Gradient
- Clean after gradient
- Reequilibration

Each block contains a number of **method variables** (name in black) with suitable default values. The values are easily changed to suit your requirements. Click on the scroll bar to see the next part of the **Variables** page.

The only values you must change in the **man_f_gr** template are for:

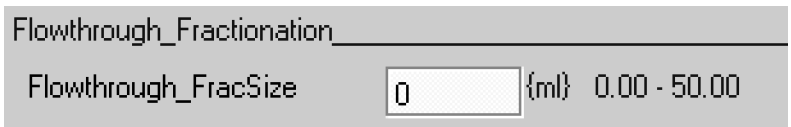
- **Empty_loop_with**, in the block Sample_Injection

  Enter a value of 5 x the volume of the sample loop to apply all the sample onto the column.



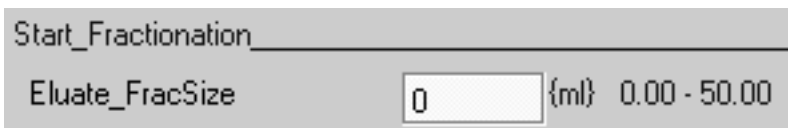
- **Flowthrough_FracSize,** in the block Flowthrough and Fractionation

  Enter a suitable fraction size. The fraction collection starts at injection and continues until gradient start. Zero means that fractions will not be collected.



- **Eluate_FracSize,** in the block Start Fractionation

  Enter a suitable fraction size. The fraction collection starts at the beginning of the gradient. Zero means that fractions will not be collected.



Comment:

Each method template is unique, but they are all built up with the same principle. Below is a description of all the blocks in the man_f_gr template

- Main

  The column selected is shown here.

- Start conditions GR

  Enter the wavelengths at which the run should be monitored.

  The UV averaging time is automatically set to a default value for the column selected.

  The pressure limit is automatically set to a default value for the column selected.

- Initial eluent condition GR

  Select the inlet for eluent A (usually A1).
  Select the inlet for eluent B (usually B1).

  Select the start concentration of eluent B (usually 0%).

  If a BufferPrep template is used (xxx_yy_AX or xxx_yy_CX, see Section 8) the pH can be entered here. Otherwise it should be zero.

  The flow rate is automatically set to a default value for the column selected.

  If you manually alter default values, and thereby exceeding the recommended values for the selected column, eventually you will get a warning when you save your method.

  Please note that the total setting range shown within brackets for the method variables is for your information only, to state the valid range for the variables.

- Column equilibration

  The number of column volumes (CV) necessary to equilibrate the column is set here. If you are using ÄKTApurifier 10 and zero is entered, no equilibration will take place.

  If you are using ÄKTApurifier 100, a sub block called System_volume_compensation is included. This block adds 8 ml of equilibration to compensate for the system gradient delay volume. This facilitates to accomplish comparable results when scaling up from small columns.

- Flowthrough fractionation

  The flowthrough fractionation starts at sample injection and continues to the start of the gradient. If not fractionated, the flowthrough will be collected in position F3 of the Outlet valve.

- Sample injection

  To apply all the sample onto the column, empty the sample loop with a volume 5 x the volume of the sample loop.

- Wash out unbound sample

  To wash out unbound sample with the starting buffer, enter the length of the wash here.

- Eluate fractionation

  By default, the fraction collection will start at the beginning of the gradient. To start eluate fractionation later than the gradient start, set Start_Frac_at to the desired ConcB value.

- Start fractionation
  Set the fraction size used during elution. Zero means that fractionations will not be collected. To end the fractionation earlier than the gradient end, set End Frac_at to the desired ConcB value.
- Gradient segment 1-3

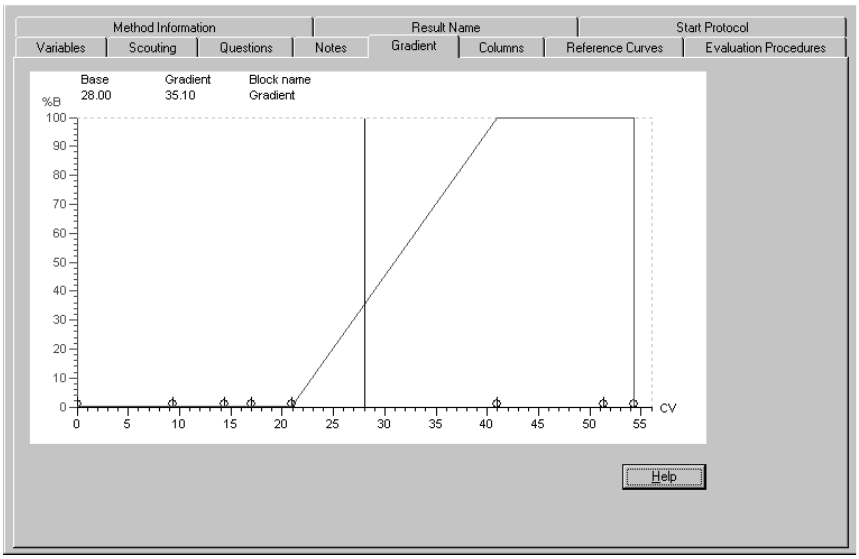
  The target concentration of eluent B and the length of the gradient is set here. A linear gradient is developed from the initial ConcB value (Start_ConcB) to the Target_ConcB_1 value (default=100%) for the duration of Length_of_gradient_1 in column volumes. Two additional gradient segments can be defined.
- Clean after gradient

  The concentration of eluent B necessary to clean the column after the separation is set here (usually 100%). The length of cleaning is also set here.
- Reequilibration GR

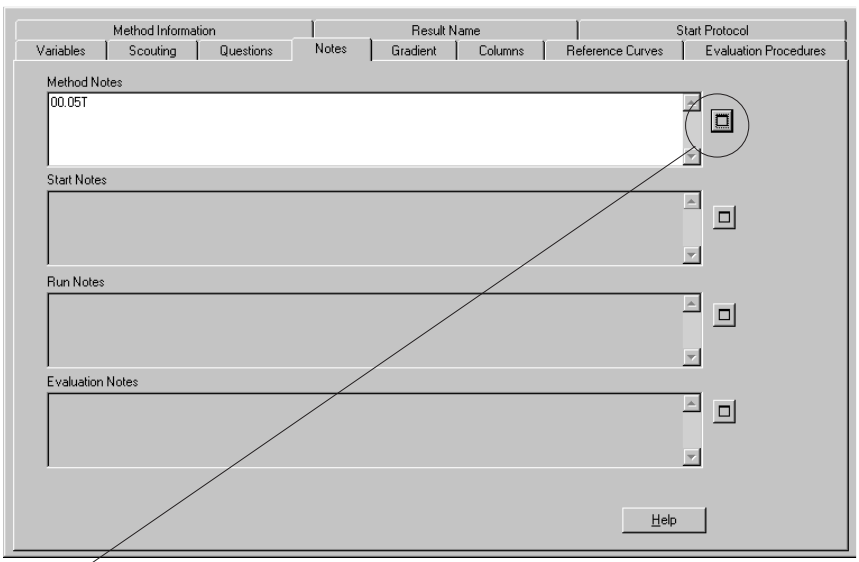
  The number of column volumes necessary to reequilibrate the column is set here. If zero is entered, no reequilibration takes place.

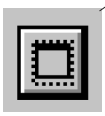
.

9 Click on the “Gradient” page to view the method graphically. The length of each block is marked at the bottom of the graph. Click in the graph. The name of the block at that position is shown in the upper part of the chromatogram. Click on the x axis to view the method in time, volume or column volumes.



10 Click on the **Notes** page.





Maximise the method notes field with the button to the right of the field. The method notes contain comments and information about the method, e.g. how sample injection, fractionation and elution are performed.

11 Select **File:Save.** Enter a name. Store the method in the directory of your choice by double-clicking on a directory. Click on **OK**.

Comment:
The method name, followed by two consecutive numbers starting with 01 will then be used as default name for the result file of your method after runs.

12 You have now created a method.

13 Click on the Main menu icon at the bottom of the screen. Your saved method appears in the window to the left.

Now you are ready to start a run. Go to chapters 4 and 5.

You can also go to chapter 8 to learn how to vary any variables systematically and automatically in repeated runs. This is know as scouting and is a convenient, easy to use function.

Comment:
If you want a more flexible method, once you are used to the basic template, select **File:New** in the method editor, and one of the more advanced templates. The template **man_f_gr** is similar to the **basic_gr** template, but allows more flexibility such as segmented gradients and flexible start of fraction collection in the gradient.

Some of the more advanced templates are named according to the following abbreviations **(xxx_y_zz)**.

Note: *Some of the functions indicated below are unic för a specific system and may not be included in the templates supplied with your system.*

The first three letters (**xxx**) identify the type of sample application used:

man = the sample loop or Superloop is filled manually with a syringe

sys = the sample is applied directly through the pump

The letter in the middle (**y**) identifies the type of fractionation used:

f = fraction collection of fixed volumes

p = fraction collection of peaks

v = valve fractionation of fixed volumes

The last two letters (**zz**) only identify the technique for which the template is written.

gf = gel filtration

ac = affinity chromatography

ax = anion exchange with BufferPrep

cx = cation exchange with BufferPrep

gr = gradient without BufferPrep

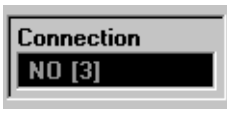
A **CIP** (Cleaning in place) template is also available. It enables automatic cleaning of the column with up to 4 different solutions.

All **xxx_y_ax** and **xxx_yy_cx** templates are pre-defined for BufferPrep (automatic buffer preparation), which allows pH scouting (see section 8 for further details).

In ÄKTApurifier 10 there are also basic templates for each one of the ..._zz templates. These templates are sligthly simplified versions of the more comprehensive xxx_y_zz versions.

4. Preparing the system for a run

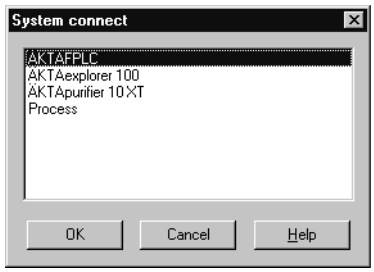
System connection



If the text "**NO**" is written in the **Connection** panel in the **Run Data** window, follow the instructions in the comment below. If not, go directly to "General system preparation".

Comment:
Before you can start a run, you must always connect to the system. Connecting means that the System Control window is set up for a particular system. If you are not connected, the text "**NO**" is written in the **Connect** panel in the **Run Data** window. Once you are connected, the text changes to "**YES**".

1 Click on the System Control icon. The System connect dialogue window appears:



2 Select a system from the list. If you are not connected to a network, only one system will be shown. Click on **OK**.

3 When connected, the text "**YES**" is written in the **Connect** panel in the **Run Data** window. You only have to connect once. If you do not select **System:Disconnect**, you will be automatically connected to the system the next time you login to UNICORN .

General system preparation

1. The correct tubings (0.25, 0.50 or 0.75 i.d. mm) for the column you intend to use must be installed. See Section 2.1 in ÄKTApurifier System Manual for an overview over columns with recommended tubings. For most columns the tubings mounted from the factory can be used.

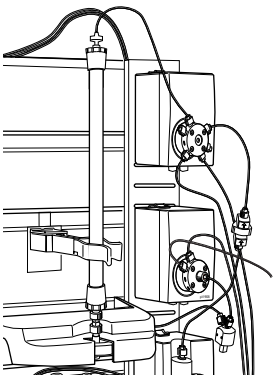
   Comment:
   If tubing with too large inner diameter are used, the peaks will become broader than necessary. If tubing with too small inner diameter are used, the backpressure from the tubing might become higher than the max. pressure for the column and the run will stop immediately after it is started.

2. Immerse inlet tubing A1 (or A2 if you changed this in the method) in buffer A and inlet tubing B1 in buffer B.

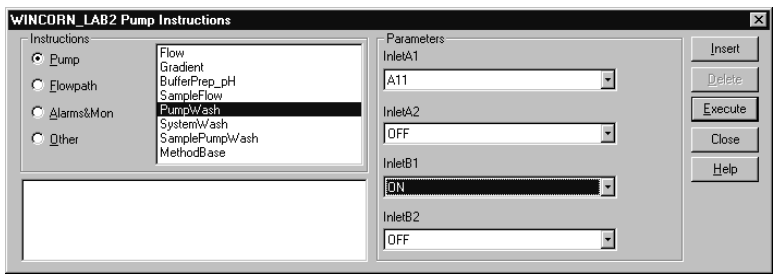
3. Put the waste tubing from port 1 of the outlet valve into a waste

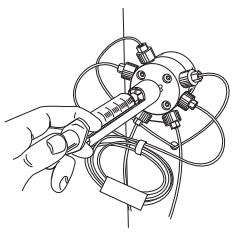
bottle. Check that the tubing from port 2 of the outlet valve is connected to the fraction collector (if used). The flowthrough will be collected via the tubing F3 from port 3 of the outlet valve, or, if you enter a value for **Flowthrough_FracSize**, in the fraction collector.

4. If there is air in the inlet tubing or if you suspect air in the pump, purge the pump with a syringe as described in section 2.8 in Pump P-900 User Manual.
5. Calibrate the pH monitor (optional) if required. Refer to Section 6.6 in UNICORN 3.0 User Manual or Section 3.6 in Monitor pH/C-900 User Manual.



6. Connect the column between port 1 of the injection valve and the top of the UV flow cell. Use a suitable length of 0.25 mm PEEK tubing (blue) supplied with your system.
7. Insert a sufficient number of tubes into Frac-900 (optional) and place the arm at the first tube.
8. Double-click on the System Control icon. Fill the inlet tubing with the correct solutions by selecting **Manual:Pump**. Then select instruction **PumpWash**. Select ON for Inlet A1 and set Inlet A2 to OFF. Select ON for Inlet B1 and OFF for Inlet B2. Click on **Execute** to fill the inlet tubing. The injection valve will switch to waste





9. Connect an injection fill port or a union luer female/1/16” male to port 3 on the injection valve and apply the sample manually with a syringe.

5. Starting a run

1 Click on the System Control icon if it is not open.

2 Select **File:Run...** Select the method to start. Click on **Run** (the method will not start yet).

3 A Start protocol appears consisting of a number of pages.

4 The first page you see is **Variables**. This is the same page you were working on in the method editor. Here you can fine tune the method before starting it. This is very convenient when repeating runs with minor adjustments.

Comment:

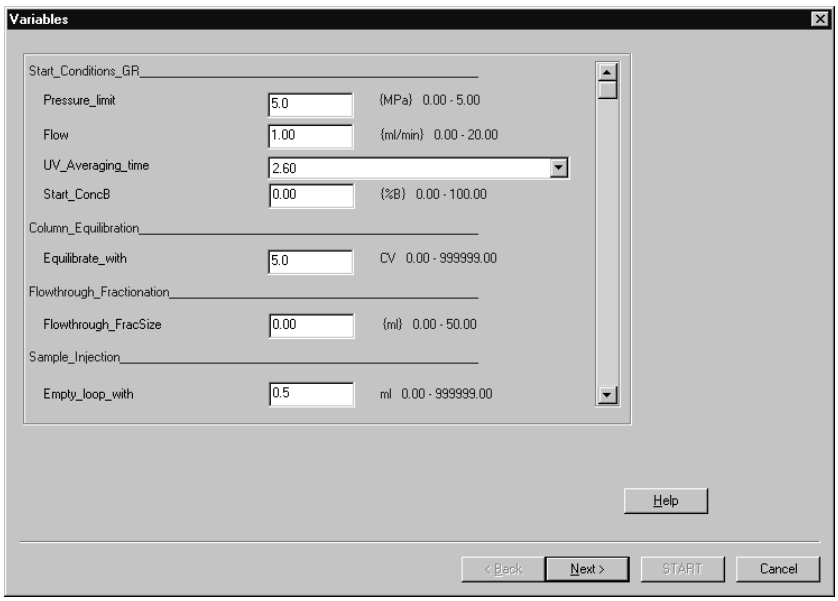
When starting run no. 2 immediately after run no. 1 with the same method but, for example, a different flow rate, you simply:

1 Click on the Run button in System Control.

2 Change the flow rate on the Variables page

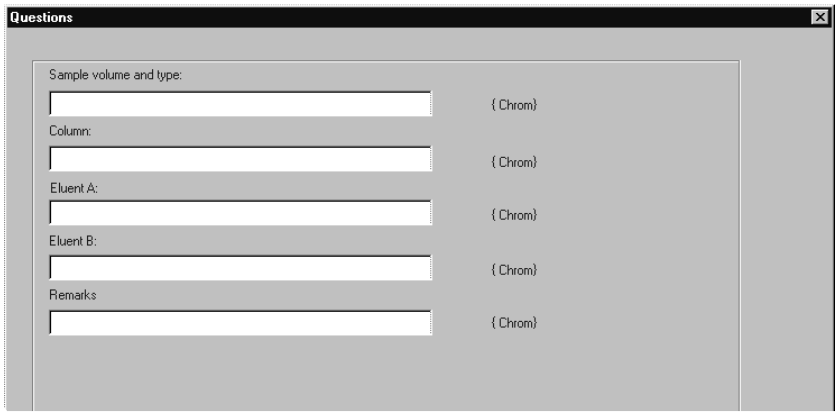
3 Continue through the start protocol by clicking on Next and then start the run.

You do not need to change the method in the Method editor



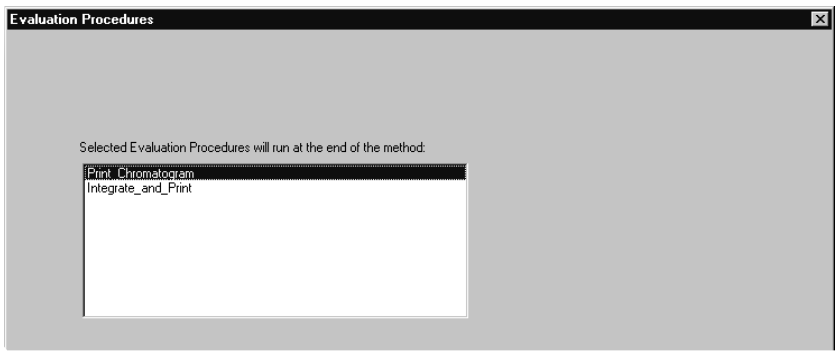
5 Go through the Variables page to check that the method is OK (this is not necessary if this was done in the method editor).

6 Click on the **Next** button at the bottom of the window; this takes you to **Questions**. Enter the answers to the questions. The answers will be saved in the result file. Some questions may have been defined as mandatory (mand). These must be answered before the run can be started.



7 Click on **Next**. This takes you to **Notes**. You can write your own comments in the Start notes.

8 Click on **Next**. This takes you to **Evaluation Procedures**. Evaluation Procedures are automated evaluation operations that are performed after the run. Mark Print_Chromatogram. The chromatogram will then automatically be printed after the run.



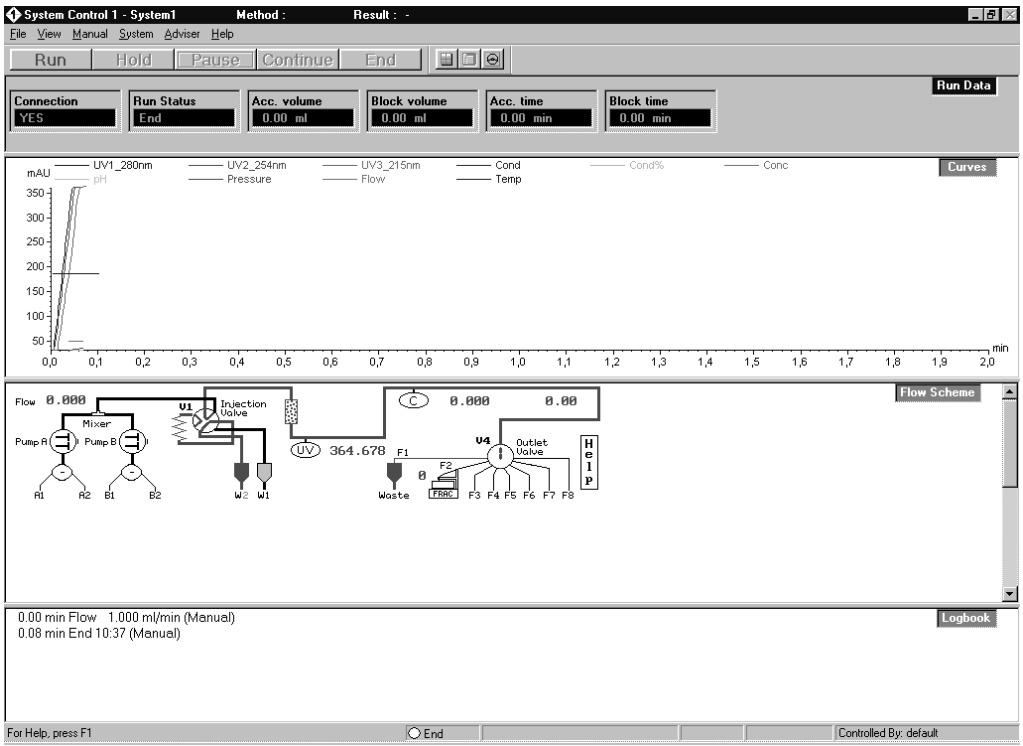
9 Click on **Next**. This takes you to **Method Information**.

Here you see information about the run. The approximate volume of buffer used (A+B) is shown as well as how long the method will take.

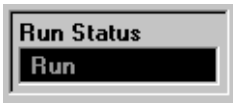
10 Click on **Next**. This takes you to **Result Name**.

Here you name the result file and define in which directory the result should be stored. A default name (the method name followed by 01) and a directory are suggested. But you can change the result name and directory (click on **Browse...**) if you so wish.

11 Click on **START**. The run will start.



6. Viewing a run

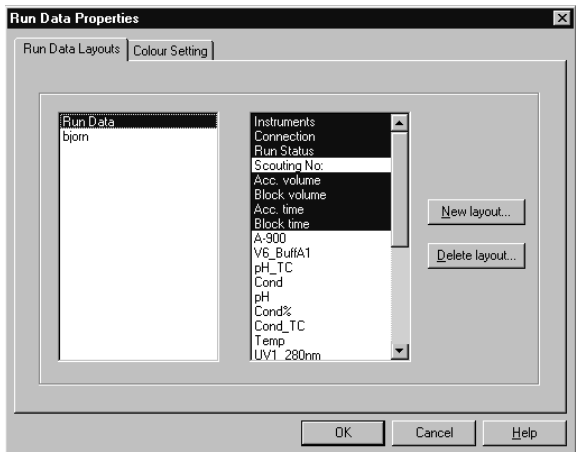


When the system pump is running, the text "**Run**" is shown in the **Run Status** panel in the **Run Data** window.

1 Select **View:Windows**. Check **Rundata, Curves** and **Logbook**. Click on **OK**.

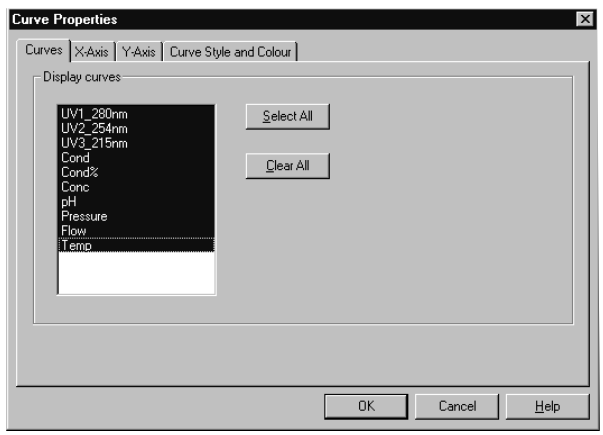
2 The **Run Data** window at the top shows current values for running parameters.

3 Position the cursor in the **Run Data** window. Click on the right mouse button and select **Properties....** Now you can select which parameters you want to see in the **Run Data** window. For eaxample, select Acc. time, Block time, flow and pressure. Click on **OK**.



4 The **Curves** window shows the curves during the run.

5 Position the cursor in the **Curves** window. Click on the right mouse button and select **Properties....** Here you can select which curves to show during the run. All curves are stored in the result file.



6 By clicking the different tabs in the **Curve Properties** window you can set the properties for the different curves. Normally the curves are scaled with auto scaling, i.e. the scale is adjusted continually to the highest and lowest values for each curve.

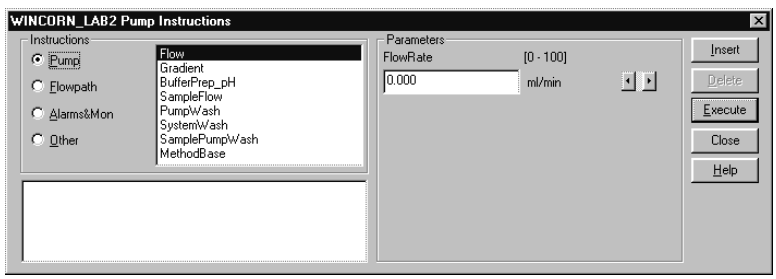
7 To fix the Y-axis scale for a curve, mark the curve, click on **Y-axis**, click on **Fixed**, and enter the max. and min. values. You can repeat this for other curves. Click on **OK**.

8 To maximise the **Curve Data** window, Position the cursor in the **Curve Data** window. Click on the right mouse button and select **Maximize.** Go back to normal size by clicking on **Restore**.

9 Click on the Y-axis scale, or click on the curve name at the top of the **Curve Data** window to shift to a scale for another curve. The colour of a curve, its Y-scale, and its name are always the same. Click on the X-axis to shift between time and volume.

10 The **Logbook** is shown at the bottom. The **Logbook** shows exactly when the instructions in the method are executed during the run. The **Logbook** is stored in the result file.

11 You can make manual changes during the run. Select **Manual:Pump**. The Instructions box is opened.



12 If, for example, you want to change the flow rate, select **Pump** and then **Flow**. Enter a new flow rate under Parameters and then click on **Execute**. The new flow rate will be used until the end of the run or until a new flow rate instruction is reached in the method. Close the box by clicking on **Close**. All manual interactions are recorded in the **Logbook**.

13 If you want to stop the run before it is finished, click on the **End** button at the top.

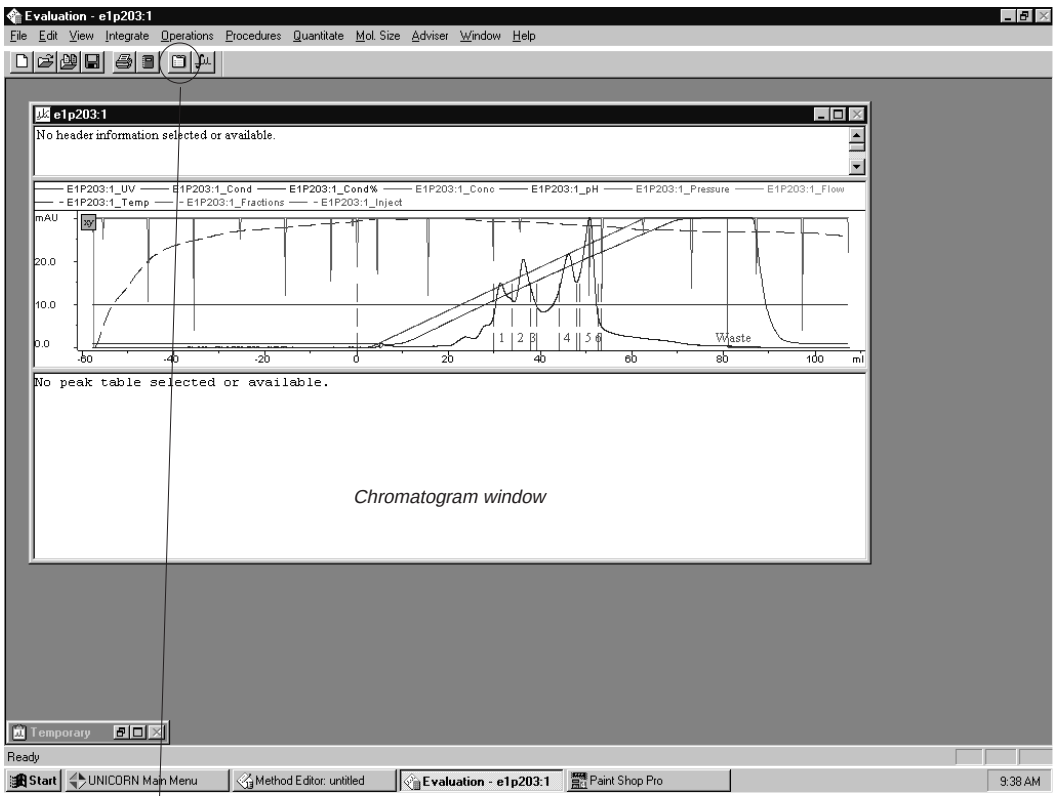
7. Viewing and printing the result

If you are satisfied with the automated printout obtained after the run (if selected), you need not alter anything described in this section. However, if you want to alter the chromatogram layout, this section will teach you the basics of the evaluation module.

Viewing

1 After a run you can view the result. Click on the Main menu icon. Double-click on a result file icon in the list to the right.

2 The **Chromatogram** window is opened automatically in the Evaluation workspace when you open a result file. The **Chromatogram** window contains all the curves. Note that the term chromatogram is used here when talking about the whole window containing all the different curves.



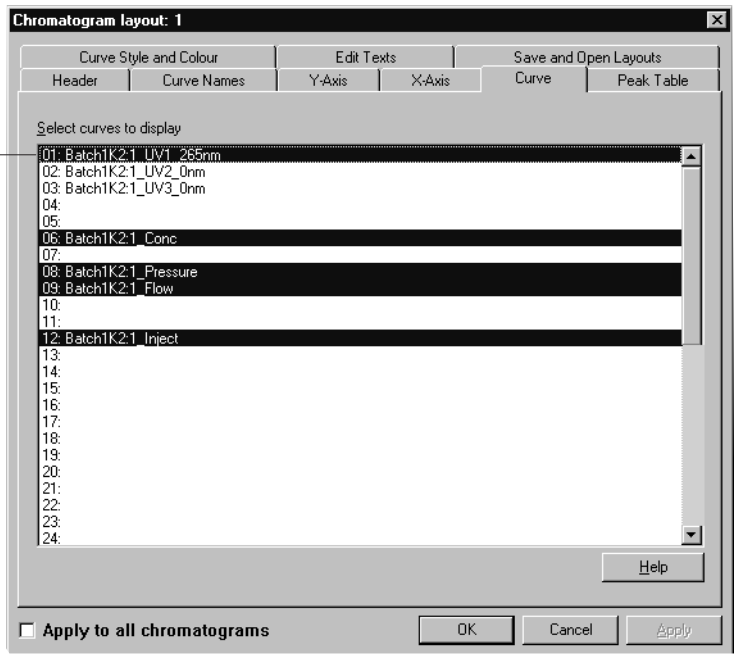
Chromatogram window

The result file from a run holds a complete record of the run, including method, system settings, curve data and run log. It is accessed by clicking the Documentation button.

Comment:
Original raw data curves can never be modified, renamed, or deleted from a result file.

3 Maximise the **Chromatogram** window by clicking on the larger square in the upper right corner.

4 All changes regarding the presentation of the curves are done in the **Chromatogram Layout** window. Position the cursor in the **Chromatogram** window. Click on the right mouse button and select **Properties....,** or select **Edit:Chromatogram layout...** to activate this window.



5 Highlight the curves to view under Curves. Curves are named as **Resultfile:1_"curve"** where a curve can be, for example, UV_wavelength, Cond, pressure ...etc. De-select all curves except, for example, the UV, Cond and Conc. curves. Click on **OK** at the bottom of the **Chromatogram Layout** window.

6 You can easily zoom in on the curves. Place the cursor in the chromatogram, click on the mouse button and holding it pressed down, move the mouse. A rectangle appears on the screen. When you release the mouse button, the part within the rectangle will be enlarged. You can zoom further on the enlarged part. Click on the right mouse button and select **Undo** or **Reset zoom** to return to the complete chromatogram.

7 Click on the Y-axis scale to change to a scale for another curve. The style and colour of a curve, its Y-scale and its X-scale can all be changed.

8 Open the **Chromatogram Layout** window again. Click on the **Y-axis** and **X-axis** tabs to set the scale for the different curves. Normally, the curves are scaled with auto scaling, i.e. the highest and lowest values for each curve set the scale.

9 To fix the Y-axis scale, mark a curve, click on **Fixed**, and enter the max. and min. values for that curve. You can repeat this for other curves.

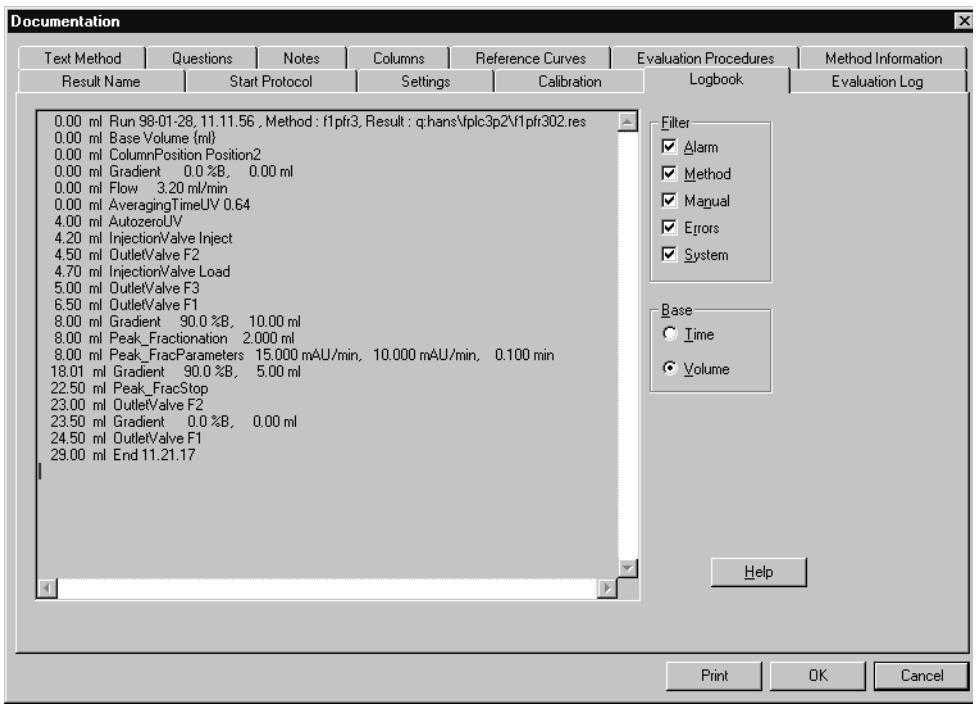
10 To fix the X-axis scale, click on **Fixed** in the X-axis field, and enter the min. and max. values for the X-axis. Click on **OK**.

11 Click on **OK** at the bottom of the **Chromatogram Layout** window to execute all the changes.

Comment:
When you have made the necessary changes in the Chromatogram layout box, they can be saved as a Layout. Click on the Save as button at the top of the Chromatogram layout box to save the layout. Give the layout a name and click on OK. Layouts can be selected in Apply layout at the top of the box and all your saved selections will apply. Saved layouts can be applied to any result file.

12 Minimise the chromatogram window by clicking on the smaller squares in the upper right corner.

13 Click on the Documentation button. A number of pages appear as in the Run Setup in the Method Editor. All documentation about the run is stored here, e.g. the method, answers to questions, variables, logbook ...etc. For example, click on the Notes and Logbook pages to check the contents. Close the **Documentation** window by clicking on the **X** in the upper right corner.

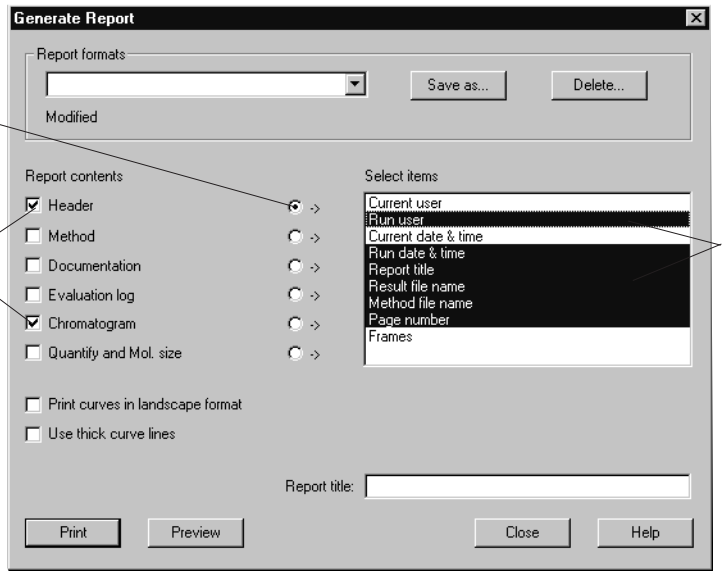


Printing and making a report

1 The quickest way to print out the chromatogram as it appears on the screen is to select **File:Print...**.

2 To include more information in the report, select **File:Report...**.

3 Under **Report contents**, select which main items to include. For example, select only **Header** and **Chromatogram**.

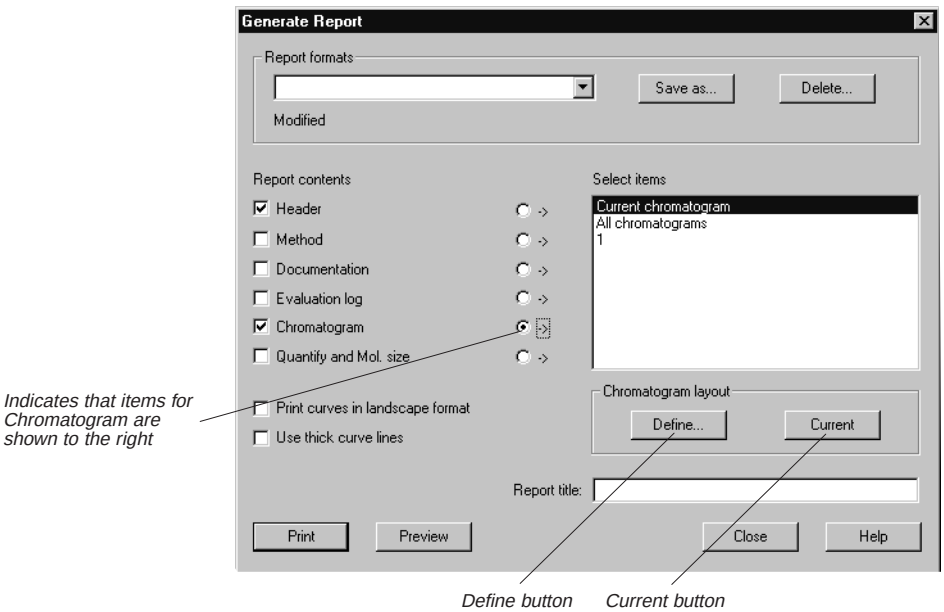


4 Check and select the contents in **Header** by clicking on the radio button beside **Header**. Select what you want to include from the panel to the right.

Comment:
You can mark items in the right-hand panel regardless of whether the corresponding Report Contents heading is selected (ticked) or not. Only the selected items for which the Report Contents headings are ticked will be printed.

5 Click on the radio button beside **Chromatogram** and select **Current Chromatogram**. The current chromatogram is the one you have open on the screen. In the next step you will select which curves to print. Remember that the term chromatogram refers to the whole chromatogram window and not to individual curves.

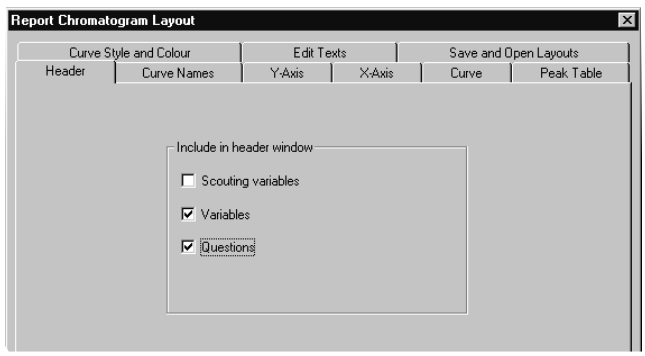
Comment:
Select **All chromatogram** if you have many chromatogram windows on the screen and want to print them all.



6a To print the current chromatogram as it looks on the screen, click on the **Current** button below the list (to get the current layout).

6b If you want to change the layout, click on the **Define** button. The **Chromatogram Layout** window is opened and you can select curves and change the layout of the chromatogram just as you did in the previous section. Mark which curves to print. Click on the **Header** button. The information selected here will be printed on top of the chromatogram. Select Variables and/or Questions. Click on **OK**. Click on **OK** at the bottom of the **Chromatogram Layout** window.

Comment:
The changes made here in the Chromatogram Layout window will only affect the printout and not the presentation of the chromatogram on the screen.



7 Click on **Preview** to view the report on the screen. Click on **Print** and the report is printed.

8. BufferPrep and Scouting

The BufferPrep function allows a buffer of any pH to be prepared on-line from four stock solutions. The pH can be varied automatically between scouting runs to find the optimal pH for the separation. A pH electrode is **not** necessary to obtain correct pH using BufferPrep. For more details about BufferPrep see Section 2.7 in ÄKTApurifier System Manual.

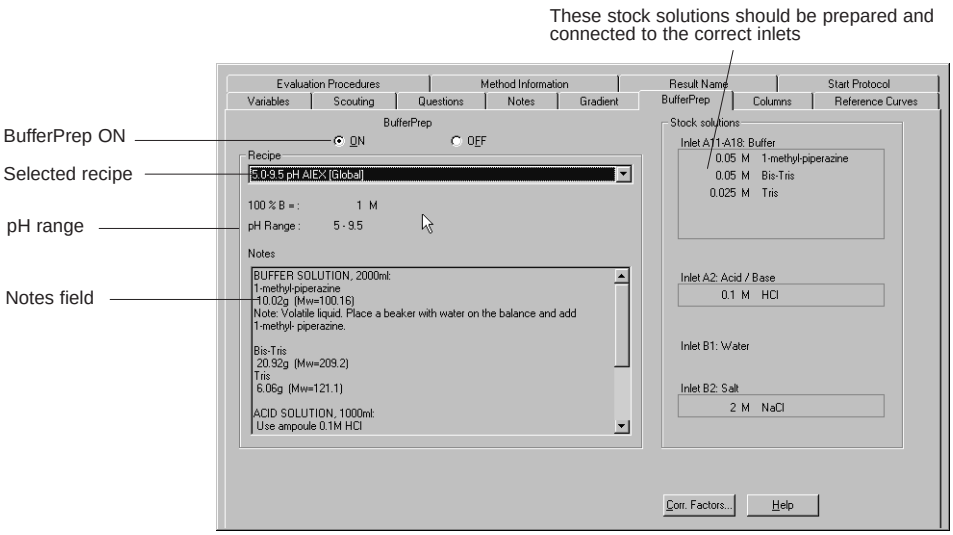
Scouting allows any run parameters, e.g. pH, to be systematically varied automatically, in repeated runs.

Below is a description of how to perform a pH scouting.

1. Select **File:New:Method** in Main menu. Select system. Then select Anion exchange as technique.
2. Select template **man_f_ax**. Select a column and click on **OK**.

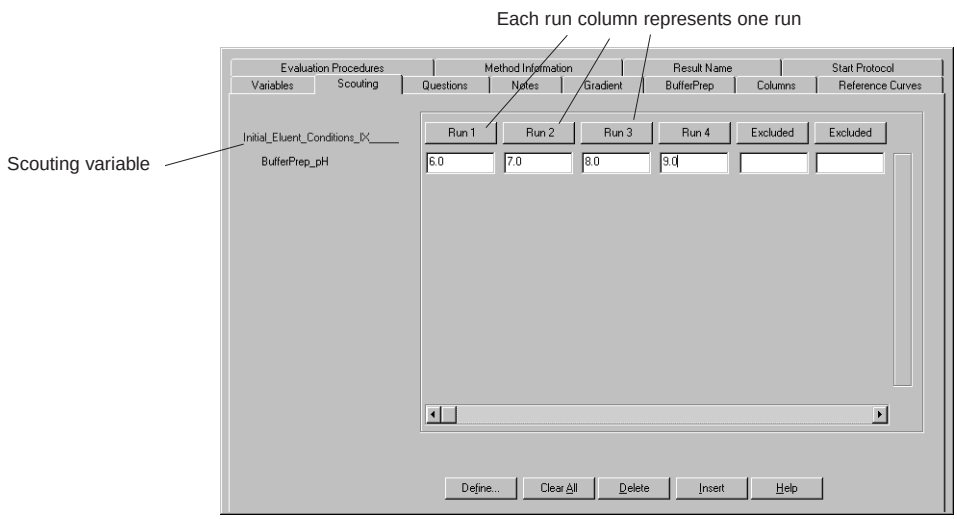
   Comment:
   BufferPrep can be used with all xxx_yy_ax and xxx_yy_cx templates, not only **basic_ax**. A special template is not required for scouting. Scouting can be performed with any template for any technique.

3. Select **View:Run setup** in the Method editor (may already be checked).
4. Click on the **BufferPrep** page.
5. The radio button is **ON** when BufferPrep is selected.



6. The recipe 5.0–9.5 pH AIEX is selected. With this buffer recipe any pH between 5 and 9.5 can be prepared on-line.
7. The required solutions and the inlets to which they should be connected are displayed to the right on the BufferPrep page. You find the correct method for preparing buffers in the Notes field. Accuracy of preparation is essential. When preparation is finished, connect them to the correct inlets.
8. Click on the **Scouting** page.
9. Click on **Define**. A list of all the variables will appear. Mark the variable BufferPrep_pH and any other variable you wish to alter, e.g. flow rate. Click on **OK**.

Comment:
Values for variables selected for scouting are greyed on the Variables page and cannot be changed there.



10. Click on any cell in the column under Run 1 in the scouting scheme. This inserts the default values for the scouting variables.
11. Make any changes you require in the variable values.

Comment:
For variables with text values (e.g. column position), double-click on the variable field and select the required value from the list that appears.

12. Click on the next run column in the next run, and click on any cell in that column, to copy the values from the preceding run, and change the values as required.
13. Repeat step 12 until you have defined all the runs you require. Use the horizontal scroll bar to see more runs.

14. Click on the buttons at the top of the scheme to toggle between **Run** and **Excluded** for the different runs. Those marked Excluded will not be run. A scouting scheme is now defined.
15. Click on the **Variables** page. Change and check the values for the same variables as in the **man_f_gr** (see point 8 in Section 3).
16. Select **File:Save**.
17. When the method is started all the runs in the scheme will be performed automatically and the set pH for each run will be prepared automatically. Each run in the scouting scheme will generate a separate result file which are all stored in a special scouting directory.
18. Prepare the system and start the run as described in section 4 and 5.

    When filling the inlet tubing with the correct solutions using the instruction **PumpWash** in **System Control:Manual: Pump**, select the correct inlet (A1) for Inlet A1 and set Inlet A2 to ON. Select ON for both Inlet B1 and Inlet B2. Click on **Execute** to fill all the inlet tubing. Click on **Close**.

    The sample should, if possible, have a pH close to the highest pH in the scouting run for anion exchange and close to the lowest pH for cation exchange.

9. Going further

Once you are used to the system and software you may want to learn more about it and its capabilities. Below is a list of operations and descriptions that you may find of interest, they are cross-referenced to other manuals in the ÄKTApurifier manual package.

To learn about	Read section
Purifying *E. coli* proteins	2 in the Method Handbook
Purifying synthetic peptides	3 in the Method Handbook
Purifying oligonucleotides	4 in the Method Handbook
Different sample application options	2.2-2.5 in ÄKTApurifier System Manual
Different fraction collection options	2.8 in ÄKTApurifier System Manual
BufferPrep details	2.7 in ÄKTApurifier System Manual
Columns and recommended tubing	2.1 in ÄKTApurifier System Manual
Changing tubing kits	A.5 in ÄKTApurifier System Manual
Calibrating monitors and pumps	6.6 in UNICORN 3.0 User Manual
Comparing chromatograms	9.4 in UNICORN 3.0 User Manual
Integrating curves	10.1 in UNICORN 3.0 User Manual
Measuring HETP and resolution	10.1.8 and 10.1.10 in UNICORN 3.0 User Manual
Exporting curves and data to other programs	10.4 in UNICORN 3.0 User Manual
Finding information about a certain menu instruction in UNICORN	Click on the Help button in the dialogue box that appears or look in the index in UNICORN 3.0 User Manual

Controlling Pump P-900, Monitor UV-900 and Monitor pH/C-900 from the dials on the instruments themselves	3 in the User Manual for each instrument, found in the binder ÄKTAdesign Components
Details about each component	See each individual manual in the binder ÄKTAdesign Components
Security features	11 in UNICORN 3.0 User Manual
Controlling the system from a remote computer	6.5 in UNICORN 3.0 User manual

November 1998

Short Instructions

When you have read through the complete “Making your first run” booklet for ÄKTApurifier, you can use these short instructions as a check list for creating a method and starting a run.

1 Select **File:New:Method** in the Main menu or click on  .

2 Select **System**, **Technique**, **Template**, and **Column**. Click on **OK**.

3 Select **File:Save** in the Method editor and give the method a name. Click on **OK**.

4 Click on 

5 Select **File:Run**. Select the method and click on **Run**.

6 The start protocol will appear. Check the method on the **Variables** page and change values as you require.

7 Click on the **Next** button and go through all the other pages.

8 On the **Evaluation procedures** page, select **Print_Chromatogram** to get a printout automatically after the run.

9 Click on the **Start** button on the last page and the run will start.

Amersham Biosciences

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